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Povidone iodine gel alcohol: A 30-second, onetime application preoperative skin preparation

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Abstract TOP

Background: Simplifying and shortening the skin-preparation application procedure is desirable for many reasons, which include labor-cost savings and improved suite utilization. A new formulation, PVP-I Gel Alcohol (PGA) that contains 5% PVP-I and 62% ethanol in gel form, was developed to achieve a shorter preparation time with a rapid and persistent efficacy on a broad spectrum of microorganisms and to minimize the potential for iodine irritation.
Method: The test methods outlined in the Federal Register, 21 CFR Parts 333 and 369, “Tentative

Final Monograph for Health-Care Antiseptic Drug Products;" Proposed Rule, 1994 (Monograph), were adapted in this study. Efficacy of PGA was evaluated, both in vitro and in vivo. The in vitro time-kill and minimum inhibition concentration tests were conducted by using 33 strains of aerobic and anaerobic gram-positive bacteria, gram-negative bacteria, yeasts, and antibiotic-resistant bacteria. In the clinical test, the inguinal and abdominal skin sites of human subjects were exposed to PGA for 30 seconds to assess the antimicrobial efficacy on normal skin flora. Betadine PVP-I scrub was tested in a 5-minute application as a control.

Results: The time-kill test showed that PGA delivered a rapid antimicrobial activity—reducing greater than 3 to 8 log microorganisms in 15 seconds in all of the 33 species of microorganisms tested. Within 30 seconds, all challenge organisms were reduced below detection level. Results of the minimum inhibition concentration test showed that PGA demonstrated an equivalent activity to Betadine control under the testing conditions. In the clinical test, PGA was effective in the reduction of greater than 3 log and 2 log of normal skin flora, respectively, in inguinal and abdominal sites in a single-step 30-second application. Bacteria levels remained significantly below the baseline for 6 hours in the primary study and for 24 hours in a secondary study. These results show that the current PGA formulation with a 30-second application delivers an efficacy equivalent to Betadine scrub in a 5-minute application and that the PGA formulation has a long-lasting effect—up to 24 hours.

Conclusion: The PGA formulation delivered rapid and persistent antimicrobial activity against a broad spectrum of bacteria both in vitro and in vivo. PGA is an effective skin-preparation formulation for use in a single-step 30-second application. (AJIC Am J Infect Control 1998;26:488-94)

United States Pharmacopeia Povidone Iodine (PVP-I) is available in various forms in household and hospital products for disinfection. Among these is PVP-I (10%) in gel form, which has been widely used in hospitals as an effective preoperative skin preparation. The gel offers localized application sites that reduce the risk of skin irritation. PVP-I in liquid form often runs and pools during preparation, which causes skin irritation and even burns.[1] The gel also forms a water-soluble film on skin when dry, which provides potential for longer-term protection.

No reports correlate the skin-preparation application time with the surgical wound infection rate or a standardized time requirement for application. The accepted application time for an iodophor preparation recommended by manufacturers and practiced by many hospitals is approximately 5 minutes. Patients are typically prepared with a scrubbing solution of 7.5% PVP-I applied in circular motion outward from the center of the surgical site, followed with a blotting or rinse, and covered with a paint of a 10% PVP-I solution.[2,3] In today's cost-conscious hospital environment, the desire exists to simplify and shorten the application of skin preparations without compromising efficacy.[4]

Alcohol is an excellent disinfectant with a rapid action against a broad spectrum of microorganisms.[5,6] However, once evaporated, the alcohol has no long-lasting antimicrobial effect. The combination of iodine and alcohol as tincture to deliver a rapid and sustained antimicrobial action is well known, but the element iodine often causes skin irritations and staining. A new formulation of PVP-I in gel form that contains 5% PVP-I and 62% ethanol described herein was developed to demonstrate rapid and persistent antimicrobial activities, both in vitro and in vivo, against a broad spectrum of microorganisms. The gel materials also serve as evaporative retardants that prolong the activity of alcohol[7] and help to form a water soluble film on the skin after drying, which provides potential persistent antimicrobial activity. The film can be easily removed by a wet towel after surgery.

Methods

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All efficacy tests conducted in this study followed the methods outlined in the Monograph.[8] The Monograph states that skin preparation must pass in vitro time-kill and minimum inhibition concentration (MIC) tests and in vivo clinical tests with human subjects. For the in vitro tests, the skin preparation must be able to control 25 microbial species listed in the Monograph, which include gram-positive bacteria, gram-negative bacteria, aerobic bacteria, anaerobic bacteria, and yeasts. In the clinical test, the skin preparation must deliver 3 log reduction of normal flora from an inguinal site with a baseline of at least 10^5 bacteria per site, and 2 log reduction from an abdominal site with a baseline of at least 10^3 bacteria per site. The bacterial counts should not exceed the baseline for 6 hours after application.

In vitro time-kill test

In addition to the 25 species listed in the Monograph, 8 strains of antibiotic-resistant bacteria of clinical significance also were included in the in vitro evaluation. Microorganisms were added to the testing materials to deliver final concentrations of about 10^5 cells/mL to 10^8 cells/mL. At the end of the exposure time, an aliquot of each of the testing materials was transferred to a neutralizer solution that consisted of 0.05% (wt/vol) sodium thiosulfate in deionized water. The sodium thiosulfate immediately stopped or “neutralized” the chemical reaction of iodine, and the neutralized solution was bioassayed by the standard pour plate method with media appropriate to specific organisms. Colonies were counted after an incubation of 48 hours under appropriate aerobic or anaerobic conditions at appropriate temperatures. The time-kill study was performed at BioScience Laboratories (Bozeman, Mont).

In vitro MIC test

All strains of microorganisms described in the time-kill test were used in the MIC test. One milliliter of microorganisms at approximate 10^6 cells/mL in culture broth was mixed with an equal volume of the testing material in a twofold serial dilution in appropriate media and incubated under appropriate aerobic or anaerobic conditions at appropriate temperatures for 16 to 24 hours or until the positive control showed growth. The last tube that exhibited no growth in the dilution series as examined by the naked eye was recorded as the MIC. The MIC test also was performed at BioScience Laboratories.

In vivo clinical study

Healthy human subjects between 18 and 70 years of age with no dermatologic diseases were screened for baseline bacterial counts that exceeded 10^5 cells per inguinal testing site and 10^3 cells per abdominal testing site. Only subjects who had bacterial counts that exceeded the requirement were qualified for the test. Twelve healthy human volunteers, both men and women, were chosen. The subjects washed with nonmedicinal cleansing agents for 2 weeks before the test. The baseline bacterial counts were again obtained at “zero minute” immediately before the test with the same qualification criteria. A standard cup-stripping method described in the Monograph was adapted to obtain bacterial counts from the skin. Briefly, a 4-cm tall sterile glass cylinder with an inner diameter of 2.2 cm was pressed against the skin, covering a site of an area of approximately 3.8 cm^2. A volume of 2.5 mL of stripping solution that contained 0.2% sodium thiosulfate in phosphate buffer (pH 7.0) was added to the cylinder at appropriate time points. The entire skin surface in the cylinder was scrubbed with a rubber policeman in a circular motion for 1 minute. The stripping solution was collected into a sterile test tube and the procedure was repeated. The repeated fluid samples were pooled and assayed for survival organisms by using the standard pour plate method. After the baseline sampling, the antimicrobials were applied to randomized skin sites in a circular motion, beginning at the center of the site and moving outward. The application time for PGA was a single step of 30 seconds. The application time for the Betadine control was 5 minutes. All other testing materials were applied in a single step of 30 seconds for comparison. After the test agents were allowed to completely air dry, a 10-minute sample was similarly taken and the test areas then were covered with a wrap of sterile gauze held in place with nonocclusive tape. The gauze dressings were renewed after each sampling. The in vivo clinical study was performed at MicroBioTest Inc (Sterling, Va).

Spore recovery test

Because PGA forms a film on skin that may inhibit the efficiency of bacterial retrieval in a clinical test, a spore recovery test was designed to validate the test methodology. Twenty microliters of *Bacillus subtilis* spores (Biological Indicator Lot No. 78-152D Baxter Healthcare Corp, Round Lake, Ill) at 3.12×10^6/mL were applied to human forearm skin covering the area of a dime and allowed to dry, followed by a gentle application of PGA to the spore-inoculated area and the peripheral area of a size about 3.5 cm in diameter. Duraprep (3M Health Care, St Paul, Minn), a water-insoluble, film-forming skin preparation was similarly applied for comparison. Spores with no cover were used as the control. The spores were retrieved by a cup-stripping technique with a 1-minute scrubbing by a cotton swab in 5 mL of a 1:1 mixture of sterile Freon TF (DuPont Chemical, Wilmington, Del) and sterile stripping solution, which contained neutralizer (0.1% sodium thiosulfate in 0.075M phosphate buffer (pH 7.0) with 0.1% polysorbate 80). The stripping solution then was collected and filtered through a 0.22 μm Millipore (Millipore Corporation, Bedford, Mass) filter, which was followed with a rinse of 10 mL sterile phosphate buffer. Both the filter and the tip of the swab were blended in a blender with 30 mL sterile 0.075mol/L Phosphate buffer (pH 7.0), which contained 0.1% sodium thiosulfate, at a high speed for 1 minute and assayed for retrieved spores with a standard quantitative technique. The spore test was performed at Baxter Healthcare Corporation.

Results

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In vitro time-kill test

Table 1 shows that in 15 seconds of contact, greater than 3 to 8 log reduction was observed in all bacteria and yeasts tested, which included the antibiotic-resistant strains of bacteria.

Table 1. Comparative study of PGA and Betadine PVP-I control in microbial time-kill study with organisms listed in the Monograph and other antibiotic-resistant species with clinical significance in 15-second and 30-second exposures

	Log reduction			
	15 s		**30 s**	
	PGA	**Control***	**PGA**	**Control***
Staphylococcus aureus (ATCC #6538)	>8.63	7.32	>8.63	>8.63
S aureus (ATCC #29213)	>5.94	>5.94	>5.94	>5.94
S aureus (ATCC #33591) M,V†	>6.07	2.11	>6.07	4.27
S aureus (ATCC #33592) G,M†	>6.15	2.24	>6.15	>6.15
S aureus (ATCC #33593) G,M†	>5.71	>5.71	>5.71	>5.71
S aureus (ATCC #33594) G†	>6.03	>6.03	>6.03	>6.03
S aureus (ATCC #43300) M†	>5.78	2.90	>5.78	>5.78
Staphylococcus epidermidis (ATCC #12228)	>5.40	>5.40	>5.40	>5.40
S epidermidis (ATCC #51624) M†	>5.75	2.86	>5.75	2.89
S epidermidis (ATCC #51625) M†	>5.33	>5.33	>5.33	>5.33
Staphylococcus haemolyticus (ATCC #29970)	>5.13	2.15	>5.13	>5.13
Staphylococcus hominis (ATCC #25615)	>5.22	1.79	>5.22	>5.22
Staphylococcus saprophyticus (ATCC#15305)	>5.55	2.26	>5.55	>5.55
Streptococcus pyogenes (ATCC #12351)	>4.51	>4.51	>4.51	>4.51
Streptococcus pneumoniae (ATCC #35088)	>4.37	3.06	>4.37	>4.37
Micrococcus lureus (ATCC #7468)	>4.56	>4.56	>4.56	>4.56
Enterococcus faecalis (ATCC #29212)	>5.98	>5.98	>5.98	>5.98
Enterococcus faecalis (ATCC #51575) G,S,V†	>6.04	4.63	>6.04	>6.04
Enterococcus faecium (ATCC #49224)	>5.61	3.84	>5.61	4.97
Acinetobacter anitratus (ATCC #49137)	>5.42	>5.42	>5.42	>5.42
Enterobacter cloacae (ATCC #13047)	>5.98	>5.98	>5.98	>5.98
Eschericia coli (ATCC #11229)	>7.66	>7.66	>7.66	>7.66
E coli (ATCC #25922)	>5.82	>5.82	>5.82	>5.82

Klebsiella pneumoniae (ATCC #27736)	>5.58	4.02	>5.58	>5.58
Klebsiella oxytoca (ATCC #15764)	>5.70	>5.70	>5.70	>5.70
Proteus mirabilis (ATCC #4630)	>6.11	>6.11	>6.11	>6.11
Pseudomonas aeruginosa (ATCC #15442)	>6.02	>6.02	>6.02	>6.02
Pseudomonas aeruginosa (ATCC #27853)	>5.81	>5.81	>5.81	>5.81
Serratia marcescens (ATCC #14756)	>5.95	>5.95	>5.95	>5.95
Bacteroides fragilis (ATCC #25285)	>5.54	4.81	>5.54	>5.54
Haemophilis influenzae (ATCC #33533)	>5.72	>5.72	>5.72	>5.72
Candida albicans (ATCC #10231)	>7.58	2.04	>7.58	5.00
Candida tropicalis (ATCC #750)	3.23	1.82	>5.54	3.35

*Betadine PVP-I (7.5%).
†Resistance in: *M*, Methicillin; *G*, gentamicin; *S*, streptomycin; *V*, vancomycin.

Within 30 seconds, all challenge organisms were reduced below detection levels. PGA showed equivalent or superior antimicrobial activity to the Betadine control in all of the organisms tested, which included the antibiotic-resistant organisms. No resistance against the antimicrobial activity of PGA by the antibiotic-resistant organisms was observed, which confirmed earlier studies of iodophore on antibiotic-resistant organisms.[9,10]

In vitro MIC tests

The MIC test demonstrated that the performance of PGA is equivalent to or better than the Betadine control in the majority of the organisms tested under the testing conditions (Table 2).

Table 2. Comparative study of PGA and Betadine PVP-I control in MIC test with the organisms listed in the Monograph and other antibiotic-resistant species

	MIC*	
	PGA	**Control†**
S aureus (ATCC #6538)	1:128	1:32
S aureus (ATCC #29213)	1:32	1:32
S aureus (ATCC #33591) M,V‡	1:64	1:64
S aureus (ATCC #33592) G,M‡	1:128	1:32
S aureus (ATCC #33593) G,M‡	1:64	1:64

S aureus (ATCC #33594) G‡	1:64	1:64
S aureus (ATCC #43300) M‡	1:64	1:64
S epidermidis (ATCC #12228)	1:64	1:64
S epidermidis (ATCC #51624) M‡	1:32	1:32
S epidermidis (ATCC #51625) M‡	1:64	1:64
S haemolyticus (ATCC #29970)	1:64	1:64
S hominis (ATCC #25615)	1:128	1:32
S saprophyticus (ATCC #15305)	1:128	1:32
S pyogenes (ATCC #12351)	1:32	1:16
S pneumoniae (ATCC #35088)	1:64	1:128
M lureus (ATCC #7468)	1:32	1:32
E faecalis (ATCC #29212)	1:32	1:32
E faecalis (ATCC #51575) G,S,V‡	1:32	1:32
E faecium (ATCC #49224)	1:64	1:32
A anitratus (ATCC #49137)	1:16	1:32
E cloacae (ATCC #13047)	1:16	1:32
E coli (ATCC #11229)	1:16	1:32
E coli (ATCC #25922)	1:64	1:32
K pneumoniae (ATCC #27736)	1:16	1:32
K oxytoca (ATCC #15764)	1:16	1:32
P mirabilis (ATCC #4630)	1:32	1:32
P aeruginosa (ATCC #15442)	1:32	1:32
P aeruginosa (ATCC #27853)	1:32	1:16
S marcescens (ATCC #14756)	1:16	1:32
B fragilis (ATCC #25285)	1:32	1:16
H influenzae (ATCC #19418)	1:16	1:32
C albicans (ATCC #10231)	1:64	1:32
C tropicalis (ATCC #750)	1:16	1:32

*Dilution from the original concentration.
† Betadine PVP-I (7.5%).
‡Resistance in: *M*, Methicillin; *G*, gentamicin; *S*, streptomycin; *V*, vancomycin.

In vivo clinical study

Fig 1 and Fig 2 show the comparative results of PGA and the Betadine control in clinical tests for antibacterial activity on human inguinal and abdominal skin sites.

Fig. 1. Antimicrobial activity of PGA 30-second application versus Betadine 5-minute application on human inguinal site.

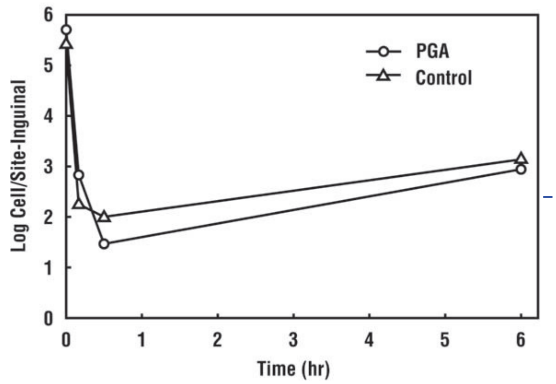

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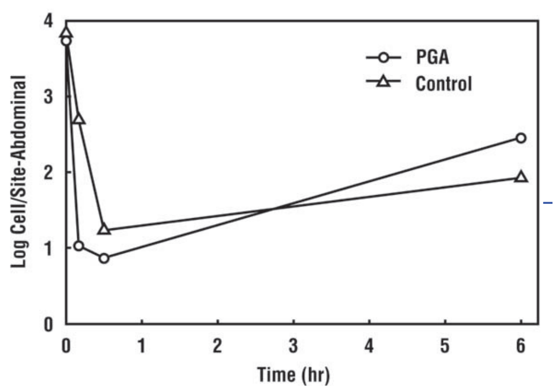
Fig. 2. Antimicrobial activity of PGA 30-second application versus Betadine 5-minute application on human abdominal site.



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After a 30-second application of PGA, approximately 3 log reduction of normal skin flora in the inguinal sites and 2.8 log reduction in the abdominal sites were observed after 10 minutes. The bacterial levels were essentially maintained and did not exceed the baseline in 6 hours. In a 5-minute application, the Betadine control delivered essentially similar results.

A separate study designed to assess the prolonged (24 hours) efficacy of PGA and the lack of prolonged effect of 62% ethanol gel without PVP-I and PVP-I (5%) gel without alcohol (Table 3 and

Table 4) showed that in a 30-second application, PGA was effective in controlling bacterial growth for at least 24 hours in both inguinal and abdominal sites.

Table 3. Antimicrobial activity of PGA at inguinal site in comparison with the active ingredients of PGA and with Betadine scrub as control

			Log reduction per inguinal site			
Sample	**Time**	**Baseline**	**10 min**	**30 min**	**6 h**	**24 h**
PGA	30 sec	6.601	ND	ND	ND	3.042
62% Ethanol gel	30 sec	5.396	2.274	1.581	0.546	ND
5% PVP-I gel	30 sec	5.396	2.272	1.372	0.686	ND
Betadine	5 min	5.339	3.053	3.352	2.247	ND

ND, Not done.

Table 4. Antimicrobial activity of PGA at abdominal site in comparison with the active ingredients of PGA and with Betadine scrub as control

			Log reduction per abdominal site			
Sample	**Time**	**Baseline**	**10 min**	**30 min**	**6 h**	**24 h**
PGA	30 sec	3.907	ND	ND	ND	1.820
62% Ethanol gel	30 sec	3.928	1.990	1.669	0.059	ND
5% PVP-I gel	30 sec	3.928	2.018	1.404	0.136	ND
Betadine	5 min	3.774	1.098	2.565	1.941	ND

ND, Not done.

However, neither alcohol gel without PVP-I nor PVP-I gel without alcohol showed effective long-lasting antimicrobial activity. These treatments showed that the organisms gradually grew to near baseline level in both inguinal and abdominal sites in 6 hours.

Spore recovery test

Table 5 shows that virtually 100% of the spores covered by PGA film were recoverable by the cup-stripping method when compared with the control.

Table 5. Spore recovery efficiency from skin-preparation–treated skin*

Treatment	CFU/site	Relative rate of recovery (%)
Control	4.74×10^4	100
PGA	4.80×10^4	101
Duraprep	0.19×10^4	4

*Number of spores applied on skin = 6.24×10^4/site. The efficiency of the recovery method was 76%.

However, only 4% of the inoculated spores covered by the water-insoluble Duraprep film was recoverable. Duraprep film could not be dissolved by the solvent Freon TF used in the test, but approximately 20% of the film was lifted off the skin as small particulates by scrubbing. The spores contained in these particulates appeared to be unable to fully grow or the results of the colony count would have reflected a higher recovery rate. These results indicate that the method validation is ultimately important in the evaluation of any skin preparation, especially for the formulations with special characteristics, such as film-forming.

Conclusion

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PGA is an effective patient preparation that is able to simplify and shorten the skin-preparation procedure. In an in vitro time-kill study, rapid bactericidal activity was shown. A prolonged inhibitive action also was observed in an in vitro MIC test. In a clinical study of a single-step 30-second application, PGA significantly reduced the number of human skin flora in the inguinal and abdominal areas and maintained bacterial counts significantly below the baseline level for up to 6 hours in the primary study and up to 24 hours in a secondary study. We conclude that PGA is an effective skin preparation that delivers in vitro and in vivo performance that exceeds the FDA proposed standards and that it offers an opportunity to simplify and shorten the skin-preparation procedures in hospitals.

The results showed that method validation is important in the evaluation of in vivo clinical tests for skin-preparation formulation of particular characteristics.

Discussion

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Formulation

A wide variety of alcohols are known to be effective against microorganisms. Different types of alcohols or alcohols at greater concentrations can be more efficacious than the current 62% ethanol used in the PGA formulation.[6] However, in a series test 62% ethanol was found most compatible and most stable with the gel materials used in the formulation (unpublished data). The concerted action of alcohol and iodine enables the formulation to lower the iodine level to 5%. The lower iodine concentration would minimize the skin irritation of iodine-sensitive patients.

In vitro tests

The most potent agents to inactivate PVP-I include free sulfur-containing amino acids, such as cysteine and methionine.[9] These agents are present in relatively high concentrations in most culture media. For the time-kill test, only a small aliquot of broth media with the bacterial culture was carried over into the iodophore solution. A larger proportion of broth media was required in the MIC test. The amount of iodine inactivated in the MIC testing system is not known; therefore, the results of the MIC test only reflect the relative activity of the testing material in comparison with the control. A 10% PVP-I releases approximately 1 ppm free iodine, which is the active ingredient against microorganisms. When PVP-I is diluted 10 to 100 times with deionized water, it releases more free iodine thus becoming more efficacious under the testing conditions.[11,12] However, under the current MIC testing conditions with overwhelming amounts of amino acids in broth media, the dilution effect was not observed. To what extent the amino acids in various types of broth media under various types of incubation conditions would affect the free iodine is not known. Because of the complexity of the testing system, data of MIC test do not always extrapolate to clinical efficacy.[13]

In vivo tests

In clinical assessment of the antimicrobial activity of a skin preparation, the method used is of paramount importance. Skin flora can be “transient or exposed” or “residential or sheltered.”[14,15] The transient or exposed microorganisms lying on the surface of the human skin are relatively easy to eliminate by the action of antimicrobials, whereas the resident or sheltered microorganisms, which stay at deeper skin sites, are more difficult to kill. In the Monograph, the FDA proposed a testing methodology to evaluate the antimicrobial efficacy on normal human skin flora on inguinal and abdominal skin areas as testing sites. This vigorous test model has become the standard guideline in the health care industry for clinical evaluation of the antimicrobial efficacy of preoperative skin preparations. Therefore, compliance with the FDA’s proposed method is an important step in product qualification. The testing of some skin preparations against laboratory grown bacteria “seeded” onto the skin is not uncommon; this practice does not reflect the reality because those bacteria are “transient” bacteria, which are relatively easy to kill.

Furthermore, the determination of in vivo efficacy depends on the effectiveness of the test method per se. The test method must: (1) be able to retrieve viable bacteria from skin, and (2) ensure the viable bacteria retrieved are not destroyed in the removal process.

PGA forms a film on the skin when dry. The film may render the test meaningless if it prevents the retrieval of the survival organisms or if the organisms are destroyed during the retrieval process. The spore validation test showed (Table 5■) that the spores covered by the water-soluble PGA film were retrievable with high efficiency, whereas the spore covered by the water-insoluble Duraprep film were recovered poorly. Use of a conventional assay method without the involvement of filtration and blending or use of a double-strength thiosulfate in the buffer system gave similar results (unpublished data).

These results indicate that the test method validation is critical to clinical studies in the evaluation of skin preparations with particular characteristics. A seemingly effective method that can accurately assess one type of skin preparation may appear to be useless in the evaluation of another. Without method validation, the data collected may lead to the belief that the skin preparation under the test is actually efficacious based on the questionable assumption that all viable cells are fully recovered.

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Publishing and Reprint Information

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Antimicrobial activity of a new intact skin antisepsis formulation
AJIC - American Journal of Infection Control
April 2003 • Volume 31 • Number 2
Antonello Russo, PharmD[a], Pier Luigi Viotti, PhD[b], Matteo Vitali, PharmD[c], Massimo Clementi, MD[d]

ABSTRACT FULL TEXT

A new, water-resistant, film-forming, 30-second, one-step application iodophor preoperative skin preparation

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ABSTRACT FULL TEXT